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New Interpretation of a Nudibranch Central Nervous System Based on Ultrastructural Analysis of Neurodevelopment in *Melibe leonina*. II. Pedal, Pleural, and Labial Ganglia

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Abstract. Electron microscopical analysis of semi-serial sections through larval stages of the dendronotid nudibranch *Melibe leonina* (Gould, 1852) revealed paired placodes of neurogenic ectoderm at the base of the foot. The location of these laterocephalic placodes corresponds to descriptions of the ectodermal site generating pleural neurons in prosobranchs. In *Melibe*, there are two sites of neuronal ingression within each laterocephalic placode. Neurons ingressing from one of these sites join the cerebral ganglia, and their initial axons extend into the cerebrobuccal connectives or run distally along the esophagus. I identify these neurons as homologues of labial ganglia neurons in archeogastropods. However, neurons derived from the second ingression site within each laterocephalic placode join the pedal ganglia. Pedal ganglia are present in hatching veligers and are linked to the cerebral ganglia by cerebropedal connectives associated with the statocyst nerves. A second connective between each cerebral and pedal ganglia appears at the onset of neuronal ingression from the laterocephalic placodes. Peripheral axons branching from this second pair of connectives are associated with laterocephalic neurons that ingress to the pedal ganglia. I argue that these are pleural neurons, meaning that the pleural ganglia in *Melibe* are uncoupled from the visceral loop.

Introduction

The nervous systems of opisthobranch gastropods have proven highly amenable to neurophysiological investigations (reviewed in Kandel, 1979; Willows, 1985–1986), and have been used to support or criticize phylogenetic theories for this taxonomically difficult subclass (Guiart, 1901; Russell, 1929; Boettger, 1954; Gosliner, 1981; Haszprunar, 1985, 1988; Schmekel, 1985). As a result, the neuroanatomy and neurophysiology of this group is the subject of a large body of literature, with the former studies extending back to the last century. It might be expected that the basic structure of opisthobranch central nervous systems (CNS) would be thoroughly understood. In fact, homologous ganglionic regions within the often highly consolidated nervous systems of opisthobranchs are essentially best guesses based on comparisons of adult neuroanatomy in primitive and derived species.

In some opisthobranchs, distinct pleural ganglia are linked by connectives to ipsilateral cerebral and pedal ganglia, and are also the first pair of ganglia along an elongate visceral loop bearing additional ganglia. This arrangement conforms to the basic design for the gastropod nervous system (reviewed by Bullock, 1965; Dorsett, 1986). However, separate pleural ganglia are not distinguishable in most extant opisthobranchs. In nudibranchs, it is always assumed that the pleurals have fused with the cerebral ganglia to form a pair of cerebropleural ganglia (see Guiart, 1901; Hoffmann, 1936; Boettger, 1954; Schmekel, 1985). This interpretation seems entirely logical because the visceral loop enters the posterior lobes of the 'cerebropleural' ganglia, and each 'cerebropleural' ganglion is often linked to the ipsilateral pedal ganglion by two connectives (Fig. 1). Presumably, the more posterior of these two connectives is the pleuropedal, and the anterior connective is the cerebropedal. However, in my previous study of gangliogenesis in the nudibranch *Melibe leonina*, I found no evidence of pleural ganglia associated with either the visceral loop or cerebral ganglia. All ganglia of the visceral loop in *Melibe* arise from ectoderm of the visceropallium and show torsional displacement, whereas

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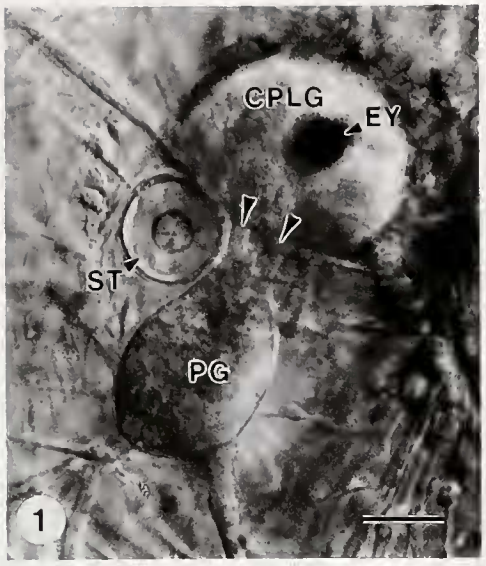


Figure 1. Light micrograph of the central nervous system of *Melibe leonina* at 48 h after metamorphic shell loss (lateral view). Traditionally, the two ganglia have been called cerebropleural (CPLG) and pedal ganglia (PG). Note the two connectives (large arrowheads) with statocyst (ST) associated with posterior connective. EY = eye. Scale 25 μm.

the pleural ganglia of gastropods are not affected by torsion.

If the pleural ganglia are not part of the visceral loop in *Melibe*, then where are they? Ultrastructural study of semi-serial thin sections through larval stages of this nudibranch, suggests that the pleural ganglia are fused with the pedal ganglia. This interpretation differs from that of an earlier developmental study by Bickell (now Page) and Kempf (1983), based on histological sections. Evidence of labial ganglia in larvae of *Melibe* was a second unexpected result. These are distinct ganglia in some archaeogastropods and a pyramidellid, but not in other adult gastropods (Fretter and Graham, 1949, 1962). The labial ganglia in larval *Melibe* fuse with the ventral side of the cerebral ganglia prior to metamorphosis.

All members of the genus *Melibe* are characterized by an oral hood, an expansion of the circumoral cephalic epidermis that is used to capture prey (Gosliner, 1987). This structure appears to be homologous to the smaller oral veil of other dendronotacean nudibranchs. Nevertheless, the large size of the oral hood may have modified gangliogenesis in *Melibe*, relative to that in other nudibranchs, particularly ganglia arising from cephalopedal ectoderm. Therefore, the generality of this new model for nudibranch CNS structure must be tested by further studies on other species.

Materials and Methods

Methods for rearing and anaesthetizing larvae of *Melibe leonina* were described by Bickell and Kempf (1983); the fixation method was that of Bickell and Chia (1979). The technique for semi-serial thin sectioning and the larval stages examined were described in the first paper of this duet (Page, 1992).

Results

As justified in the previous paper (Page, 1992), ganglionic primordia were identified by the locations of their neurogenic ectoderm, as compared to those described for the ganglia of prosobranchs. Trajectories of associated connectives, commissures, and peripheral axon tracts were also very useful for identifying ganglia derived from posttrochal cephalopedal ectoderm. The sketch in Figure 2 shows approximate positions of neurogenic ectoderm for pedal, pleural, and labial ganglia in veligers that have completed mantle retraction (approximately midway through the larval phase).

Pedal ganglia and cerebropedal connectives

In hatching veligers, an axon tract emerges from the ventral aspect of each cerebral ganglion, extends past the ipsilateral statocyst, and associates with a small cluster of subepidermal pedal cells. The axon tracts are the left and right cerebropedal connectives and the bilaterally symmetrical cell clusters within the foot are anlagen of the pedal ganglia (Fig. 3). The statocyst nerves, which each leave their respective statocyst in company with a blind, ciliated static duct, combine with the ipsilateral cerebropedal connective as the two join the cerebral ganglion (Fig. 4). Therefore, the cerebropedal connectives can be

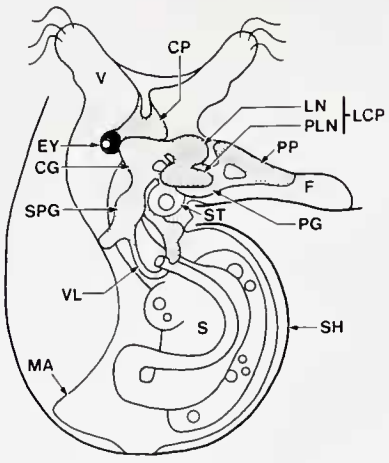


Figure 2. Right lateral view of *Melibe leonina* larva shortly after mantle retraction showing developing CNS (scant stippling) and approximate locations of neurogenic ectoderm (dense stippling). The laterocephalic placodes (LCP) include zones of ingression for both labial and pleural neurons (LN and PLN, respectively). CG = cerebral ganglion; CP = cephalic plate; EY = eye; F = foot; MA = mantle fold; PG = pedal ganglion; S = stomach; SH = shell; SPG = supraintestinal ganglion; ST = statocyst; V = velar lobe; VL = visceral loop.

identified in all larval and post-larval stages by their association with the statocysts and statocyst nerves. In young larvae, the cerebropedal connective is the only axon tract extending between each cerebral and pedal ganglion. In post-metamorphic animals, the cerebropedal connective becomes the more posterior of two connectives extending between each of these ganglia (Fig. 1).

Within each pedal ganglion of hatching veligers, the fiber tract of the cerebropedal connective breaks up into small bundles (Fig. 5) that extend independently to the overlying pedal ectoderm.

During subsequent development, the pedal ganglia enlarge greatly by addition of cells ingressing from paired ectodermal placodes (pedal placodes) extending down each side of the ventral surface of the foot. Sites of cellular ingression appear to be restricted to where the peripheral axon tracts run into the pedal ectoderm (Fig. 6). These peripheral tracts become the anterior, medial, and posterior pedal nerves of post-metamorphic animals.

The left and right pedal ganglia become connected by a pedal commissure by mantle retraction stage, and a parapedal commissure is distinguishable in histological sections of metamorphic animals.

Pleural ganglia

In 6-day-old larvae, a pair of thickened ectodermal placodes has appeared where the two sides of the foot merge with the head, just beneath the origin of the velar lobes and lateral to the mouth and statocysts (Figs. 7, 8, 9). The placode on the right side is larger than that on the left, but the positions of the two are bilaterally symmetrical (Fig. 7). These laterocephalic placodes are immediately proximal to the pedal placodes that give rise to pedal ganglion neurons, and the right laterocephalic placode is separated from the right pallial placode by the nephrocyst pores. Therefore, the laterocephalic placodes are components of post-trochal cephalopedal ectoderm.

On each side, the visceral loop and cerebropedal connective travel past the ipsilateral laterocephalic placode, shortly after these fiber tracts emerge from their respective cerebral ganglion (Fig. 9). However, neither tract associates in any way with the adjacent laterocephalic placode in 6-day-old larve. Placodal ectodermal cells do not begin to ingress until shortly before mantle retraction.

Sections through larvae at 6 days after hatching suggest that the base of each laterocephalic placode is constricted by underlying muscle fibers (Fig. 9). The adjacent membranes of both the muscle and placodal cells are thrown into folds (Fig. 10), and the two are connected by numerous adherens junctions. Furthermore, the manner in which the ectodermal cells are contorted suggests that their basal ends are being pulled toward the pedal placode located more distally along the foot (Fig. 8).

Each laterocephalic placode eventually delivers neurons to the developing CNS from two separate sites of cellular ingression. Neurons arising from the more ventro-medial of these sites fuse with the ipsilateral cerebral ganglion; these are homologues of labial ganglia (see following section of Results). Neurons ingressing from the second, more lateral site fuse with the ipsilateral pedal ganglion and represent the pleural ganglion on each side. Onset of cellular ingression from the laterocephalic placodes is correlated with the appearance of the second connective extending between each cerebral and pedal ganglion. I identify these as the cerebropleural connectives; they lie anterior to the previously formed cerebropedal connectives. The positions of the labial and pleural ganglia in *Melibe* larvae, relative to other components of the CNS, is shown in Figure 11.

For an unknown reason, ingression of cells from the two laterocephalic placodes does not occur symmetrically on both sides of the larva. Labial neurons begin ingression on the left side before ingression starts on the right side. Conversely, the timetable for ingression of pleural neurons on the right side may be slightly ahead of that on the left side, although this appearance may actually result from fewer pleural neurons ingressing from the left compared to the right placode (the left laterocephalic placode is smaller than the right). Nevertheless, in larvae examined after full mantle retraction, concurrent ingression of both labial and pleural neurons is evident on both sides.

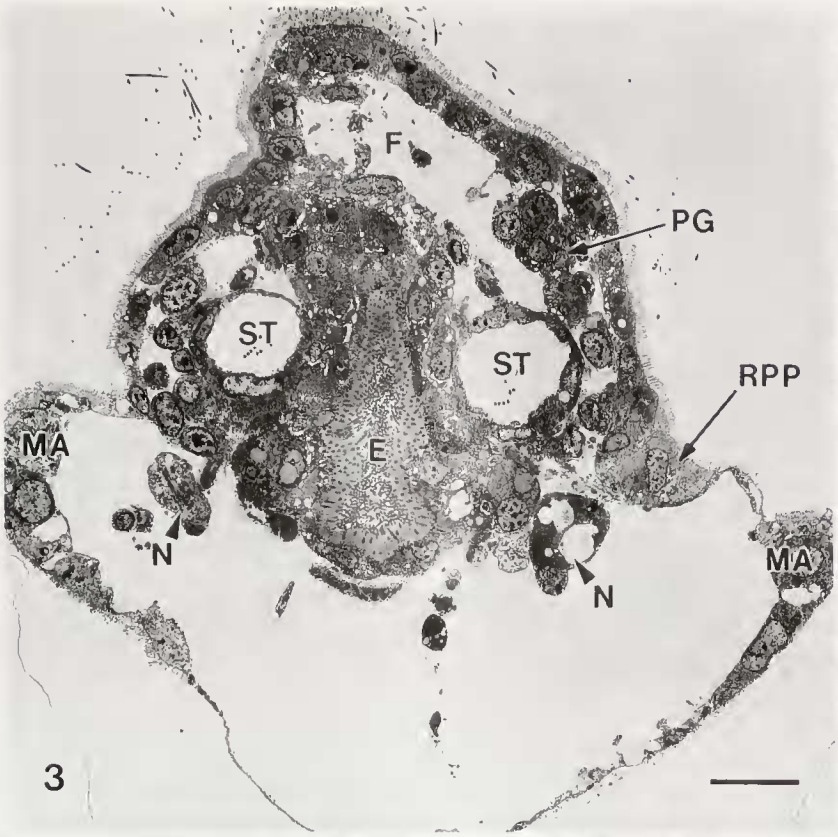
The series of micrographs in Figures 12 to 15 show an early stage in the development of the right pleural ganglion and reveal its relationship to the cerebropleural connective

Figure 3. Low magnification electronmicrograph of slightly oblique cross section through newly hatched larva of *Melibe leonina* showing right pedal ganglion (PG; enlarged in Fig. 5) located distal to the statocysts (ST) within the foot (F), and the upper extremity of the right pallial placode (RPP). E = esophagus; MA = shell secreting cells of mantle; N = nephrocysts. Scale 10 μm.

Figure 4. Cross section through base of right cerebral ganglion (CG) in newly hatched larva showing close association between statocyst nerve (SN) and cerebropedal connective (CPC) where they merge with the cerebral neuropile (NP). SD = static duct. Scale 3 μm.

Figure 5. Pedal ganglion (PG) of newly hatched larva beneath ectoderm of foot (F). Arrows indicate three small axon bundles. Scale 3 μm.

Figure 6. Portion of pedal ganglion (PG) and overlying pedal placode (PP; label is on mitotic cell) at mantle fold hypertrophy stage. Ingressing pedal neurons (arrowheads) are associated with peripheral axon tract (arrow). Scale 3 μm.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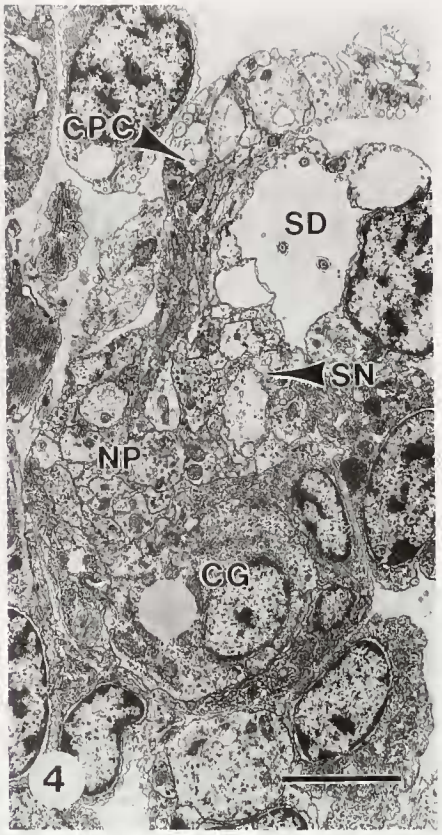
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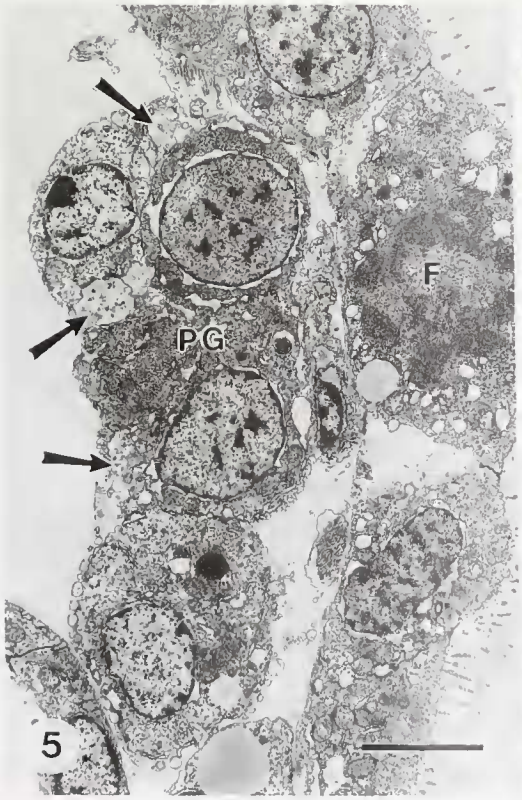
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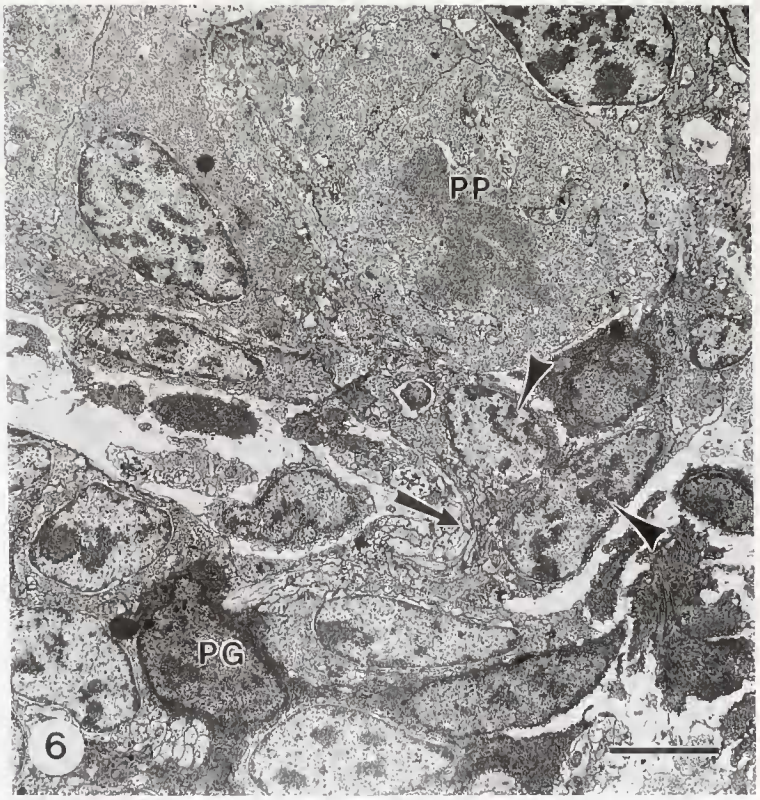
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and pedal ganglion. Figure 12 passes through the laterocephalic placode adjacent to the statocyst. An enlarged area of this section (Fig. 13) reveals that the right cerebropedal connective, associated with the statocyst nerve, has emerged from the cerebral ganglion, whereas the fiber tract of the cerebropleural connective is still associated with the antero-ventral extremity of the cerebral ganglion. In Figure 14, the cerebropleural connective is leaving the cerebral ganglion and the fiber tract is bifurcating. In Figure 15, a bulge of neurons is evident on the anterior border of the pedal ganglion; this is the anlage of the right pleural ganglion. The cerebropedal connective is entering the pedal ganglion and the main body of the cerebropleural fiber tract is deflecting medially. Axons forming the other branch of the previous bifurcation of the cerebropleural connective are emerging from the pleural ganglion at two sites. These peripheral axon tracts extend to the adjacent laterocephalic placode and are associated with ingressing pleural neurons (Fig. 15). Later in larval development, at least one of the two peripheral axon tracts extending to each laterocephalic placode is joined by axons that appear to arise from the cerebropedal connective.

The cerebropleural connective lengthens after its initial formation and the boundary between pleural and pedal ganglia is indistinguishable in late stage larvae.

Labial neurons have not begun to ingress from the right laterocephalic placode at onset of mantle retraction, although this process is evident on the left side as described in the following section.

Labial ganglia

The series of micrographs shown in Figures 16 to 19 were taken from the left side of the same larva shown in Figures 12 to 15. They illustrate the early formation of the left labial ganglion from an ingression site within the laterocephalic placode that is distinct from the ingression site for pleural neurons.

In Figure 16, the cerebropedal connective has left the cerebral ganglion but the cerebropleural connective is still within the antero-ventral extremity of this ganglion. Immediately after the cerebropleural connective has left the cerebral ganglion, the ganglion acquires a prominent lateral extension of neurons (Fig. 17) that is continuous with the laterocephalic placode (Fig. 19). These neurons are clearly fusing with the cerebral ganglion, and evidence from a slightly older developmental stage (see below) indicates they are homologues of the labial ganglia found in archeogastropods.

Figures 17 and 18 show a second site of neuronal ingression from the laterocephalic placode, located slightly beneath and lateral to that for labial neurons. These are ingressing pleural neurons associated with a small tract of axons that branched from the cerebropleural connective. The pleural neurons are extending toward the pedal ganglion, but unlike the right pedal ganglion of this larva, the left pedal ganglion has not yet acquired a prominent bulge of pleural neurons.

The micrographs in Figures 20 to 25 show the trajectories of initial axons elaborated by labial ganglion neurons on the left side. At the time of fixation, this larva had completed mantle retraction and mantle fold cells had begun proliferation prior to forming the definitive dorsal epidermis. Figure 20 passes through an area comparable to that of the younger stage shown in Figure 16. In addition to the cerebropedal and cerebropleural connectives, the cerebrobuccal connective is prominent and extends to a thickening of the ventral esophageal wall that is neurogenic ectoderm for the left buccal ganglion. A subsequent section through the base of the cerebrobuccal connective (Fig. 21) shows a group of axons that originated from the fiber tract of the cerebrobuccal connective, plus two other axon tracts that both originated from labial neurons (Figs. 22, 23). One of the labial axon tracts merges with the tract extending from the cerebrobuccal connective (Fig. 21), whereas the other labial tract extends directly into the cerebral ganglion (not shown). The combined labial and cerebrobuccal axons form a peripheral nerve that extends distally along the wall of the esophagus (Fig. 22), forming synapses on esophageal cells just inside the mouth (labial nerve; Figs. 24, 25).

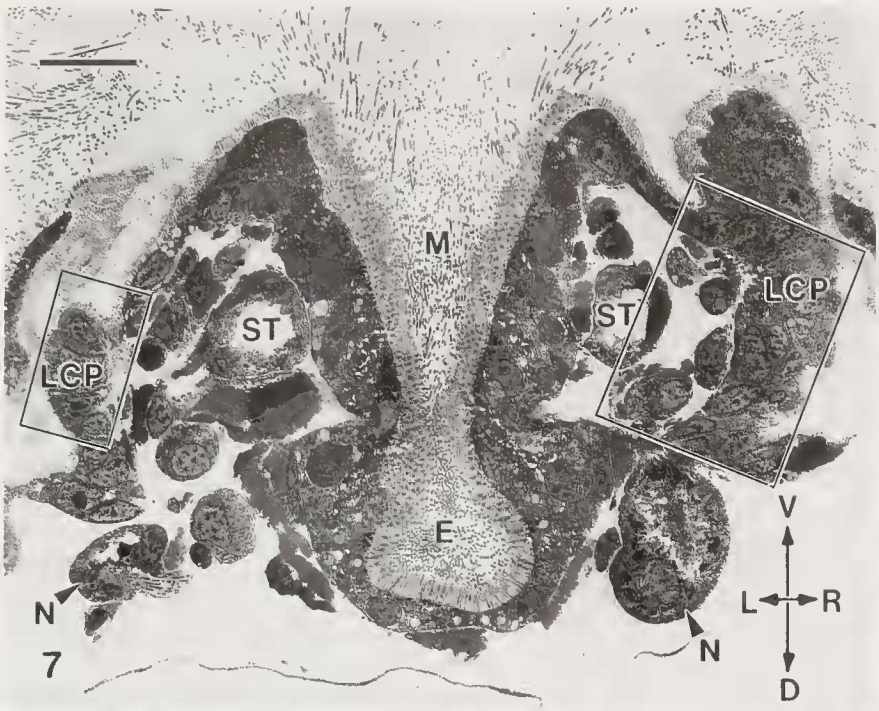
Animals sectioned immediately after metamorphic shell loss show a prominent plug of neuronal cell bodies within the ventro-lateral aspect of each cerebral ganglion, anterior to the connectives. This corresponds to the site where labial neurons have joined the cerebral ganglia. The labial

Figure 7. Low magnification electron micrograph of a cross section through a 6-day-old larva. Boxed areas contain left and right laterocephalic placodes (LCP), which are enlarged in Figures 8 and 9, respectively. Note size difference between two placodes. Orientation arrows: L = left; R = right; D = dorsal; V = ventral. E = esophagus; M = mouth; N = nephrocysts; ST = statocysts. Scale 10 μm.

Figure 8. Left LCP. Note how bases of placodal cells are flexed in direction of foot (arrow). M = muscle fiber. Scale 3 μm.

Figure 9. Right LCP. Note adjacent cerebropedal connective (CPC) and visceral loop (VL) emerging from cerebral ganglion. Muscle fibers (M) underly placodal cells. Asterisk marks position of nephrocyst duct in slightly deeper section. MF = right mantle fold. Scale 3 μm.

Figure 10. Base of right LCP. Arrowheads indicate processes from underlying muscle fiber (M) extending to convoluted basal lamina of placodal ectoderm. Scale 1 μm.



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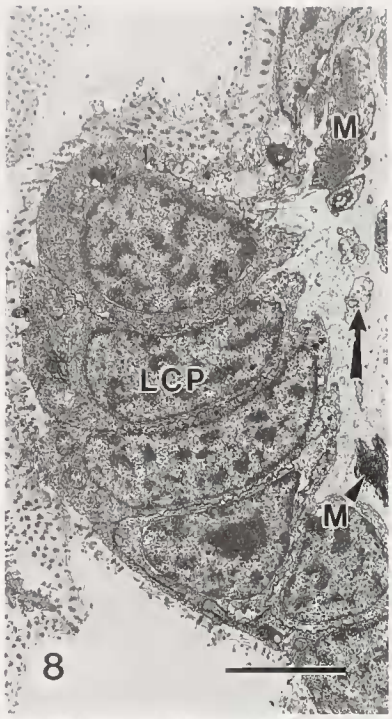

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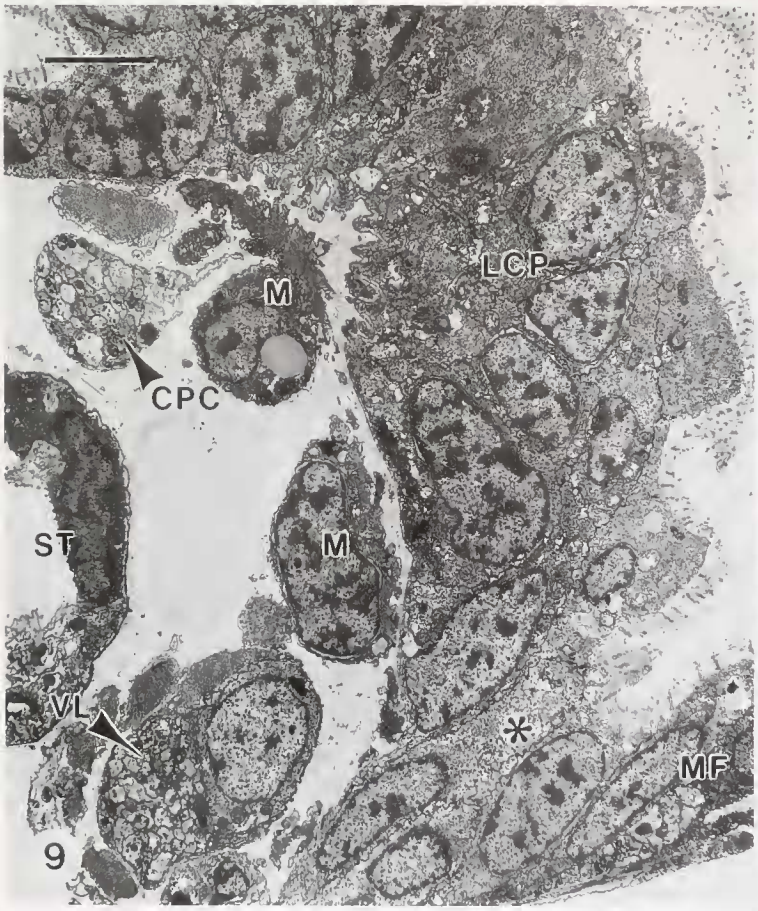

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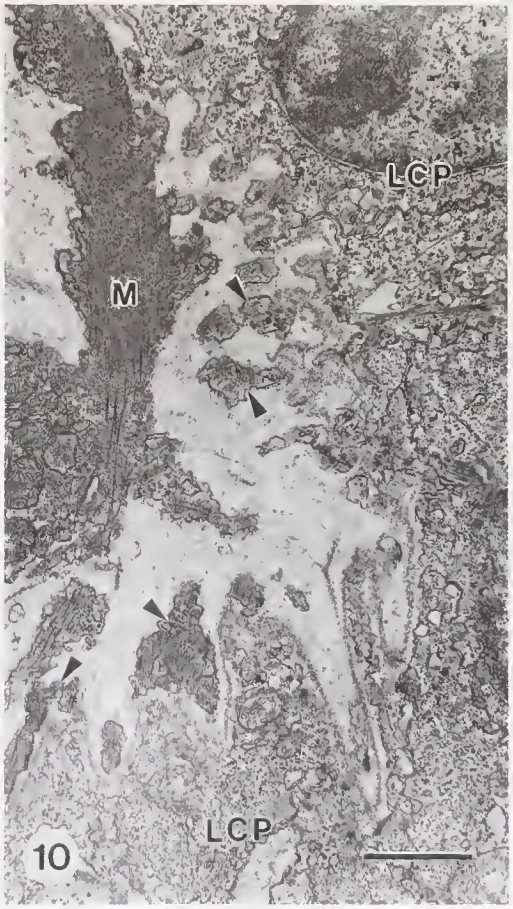

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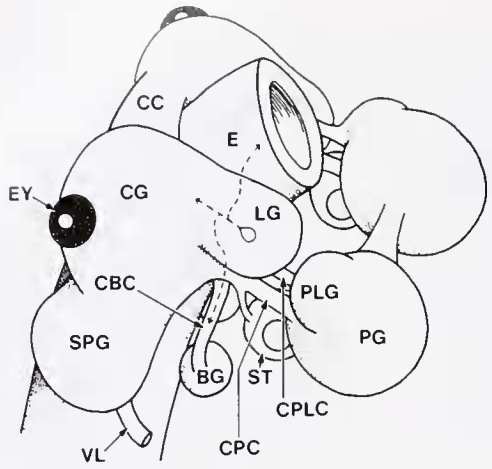

Figure 11. Reconstruction of cephalopedal ganglia and supraintestinal ganglion in larva of *Melibe leonina*, right, ventro-lateral view. Ganglia and connectives labelled on right side only. For clarity, rhinophoral ganglia are omitted and connectives over the statocysts are elongated. Broken lines show initial axon tracts of labial neurons (all tracts do not necessarily arise from single neurons). BG = buccal ganglion; CBC = cerebrobuccal connective; CC = cerebral commissure; CG = cerebral ganglion; CPC = cerebropedal connective; CPLC = cerebropleural connective; E = esophagus; EY = eye; LG = labial ganglion; PG = pedal ganglion; PLG = pleural ganglion; ST = statocyst; VL = visceral loop.

nerve becomes the nerve labelled C1 in adult *Melibe* by Hurst (1968). This nerve innervates the oral tube and lips.

Discussion

Identifications of ganglia

Pedal ganglia. The pedal ganglia in *Melibe leonina*, like those in other gastropods, arise from proliferative ectoderm along ventro-lateral zones of the larval foot. Ingressing pedal neurons are associated with axons that extend from the intraganglionic fiber tract of each cerebropedal connective, to the overlying neurogenic ectoderm of the pedal placode. These axon tracts become peripheral nerves after metamorphosis. A similar association between ingressing neurons and peripheral axon tracts was observed for pleural and visceral ganglia.

In *Aplysia californica*, the number of neurons within all central ganglia continue to increase after metamorphosis (Cash and Carew, 1989), and results of McAllister *et al.* (1983) and Hickmott and Carew (1991) suggest that added neurons come from the body wall. Jacob (1984) and McAllister *et al.* (1983) proposed that ingressing neurons in larvae and juveniles of this species migrate along connective tissue strands or muscle fibers to reach their definitive locations within the CNS. Alternatively, observations on *Melibe* larvae suggest that ingressing neurons may be guided to developing ganglia by migrating along peripheral axon tracts. After metamorphosis, these tracts continue to connect the CNS with the often distant body wall, and are therefore ideally suited to guide later ingressing neurons to appropriate central ganglia.

Pleural ganglia. My identification of pleural ganglia in *Melibe* larvae, and their developmental fate, is probably the most controversial part of this analysis and therefore requires detailed justification.

Histological studies of neurogenesis in prosobranchs and pulmonates have shown that the ectodermal placode giving rise to pleural neurons is post-trochal (Smith, 1935; Crofts, 1937; Régondaud, 1961, 1964; D'Asaro, 1969; Cumin, 1972; Honegger, 1974; Guyomarc'H Cousin, 1974; Demian and Yousif, 1975; Raven, 1975). Nevertheless, Tardy (1970, 1974), studying the nudibranch *Aeolidiella alderi* by means of histological sections, claimed that both cerebral and pleural neurons are derived from pre-trochal cephalic plate ectoderm, so that cerebral and pleural ganglia are fused from the outset. Smith (1967) suggested the same for the cephalaspid *Retusa obtusa*, and Jacob (1984) also claimed a common site of origin for cerebral and pleural ganglia in *Aplysia californica*.

If previous studies have correctly identified a post-trochal ectodermal ingression site for pleural neurons in prosobranchs and pulmonates, then I reject the notion of a common origin for cerebral and pleural neurons in opisthobranchs. Conklin's (1897) monumental study of cell

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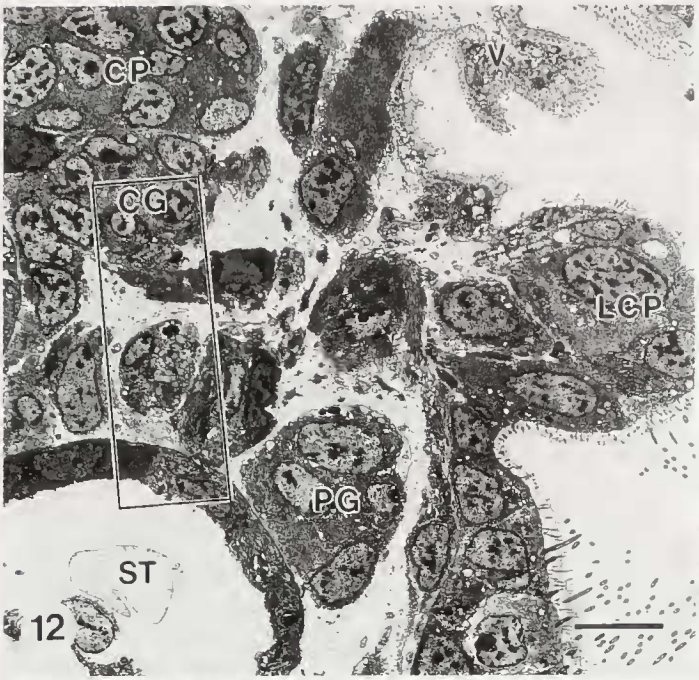
Figures 12 to 15. Series of frontal sections through right laterocephalic placode (LCP) showing cerebropleural connective and pleural ganglion.

Figure 12. Right LCP immediately beneath velar lobe (V) and opposite the statocyst (ST). Boxed area enlarged in Figure 13. CG = cerebral ganglion; CP = cephalic plate; PG = periphery of pedal ganglion. Scale 5 μm.

Figure 13. Detail from Figure 12 showing cerebropedal connective (CPC) with associated statocyst nerve (SN); cerebropleural connective (CPLC) is still within cerebral ganglion (CG). Scale 2 μm.

Figure 14. Same area in slightly deeper section showing cerebropleural connective (CPLC) emerging from cerebral ganglion (CG). Arrow indicates axons branching from CPLC. Scale 2 μm.

Figure 15. Subsequent section showing anlage of pleural ganglion (PLG) perched atop pedal ganglion (PG). Note fiber tracts of cerebropedal and cerebropleural connectives (CPC and CPLC, respectively). Axons that branched from CPLC in Figure 14 are emerging from pleural ganglion at two points (arrows). Note pleural neurons (PL) ingressing from laterocephalic placode (LCP). CG = cerebral ganglion; ST = statocyst. Scale 2 μm.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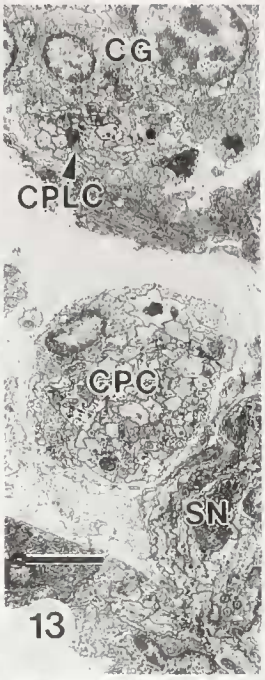
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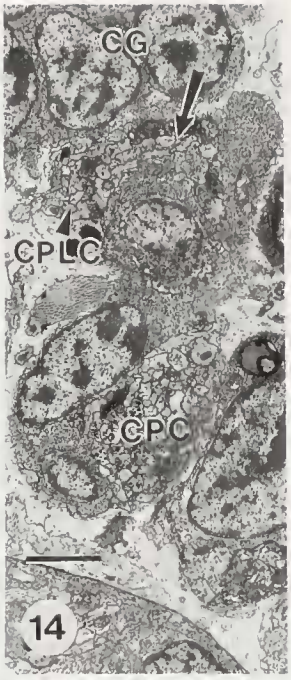
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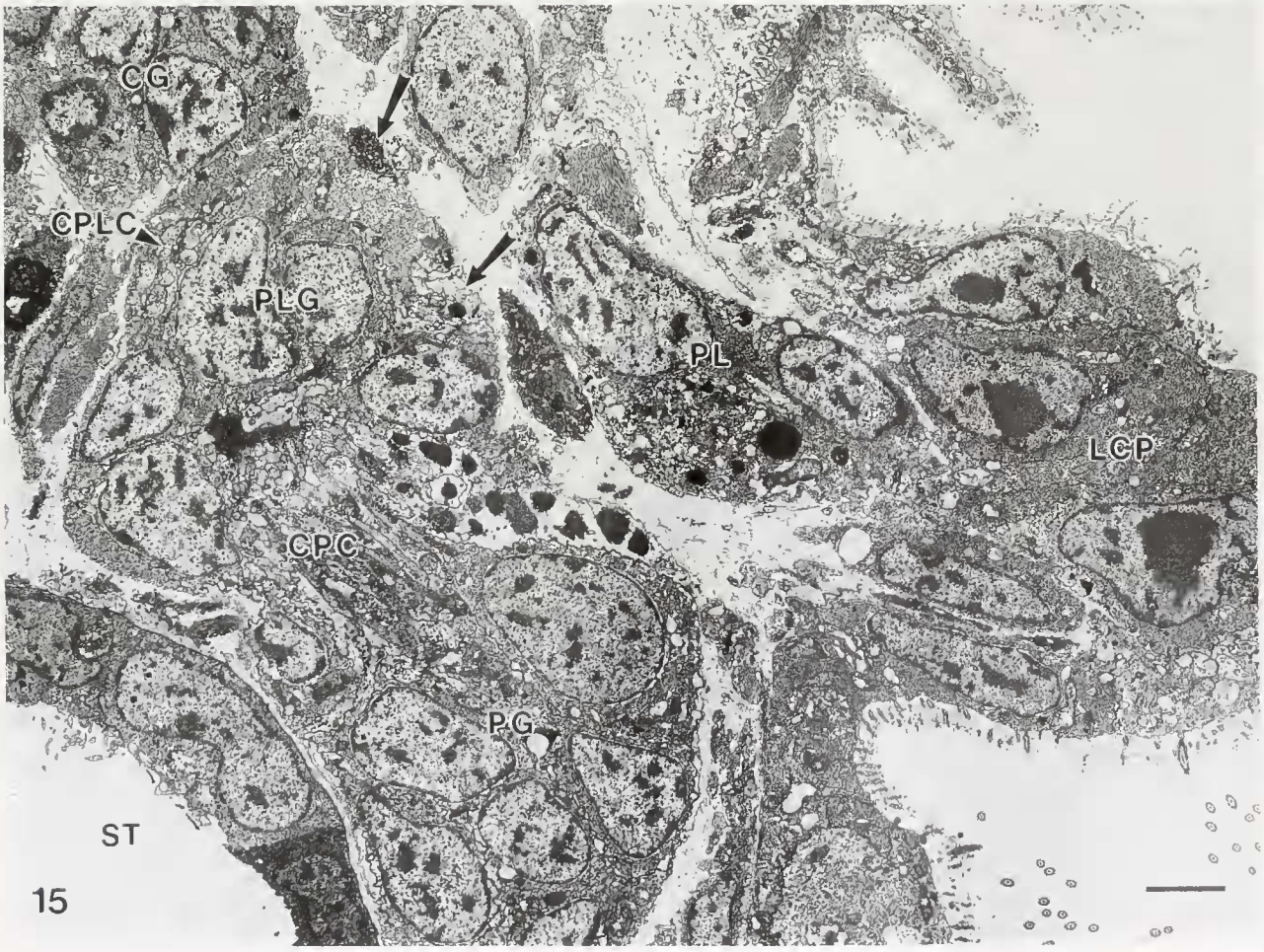
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lineage in the prosobranch gastropod, *Crepidula*, showed that the cephalic plates are derived from the first quartet of embryonic micromeres, whereas post-trochal ectoderm is derived from subsequent micromere quartets. This has been confirmed by many other studies (reviewed by Raven, 1958; Verdonk and van den Biggelaar, 1983), including Casteel's (1904) cell lineage study on the nudibranch *Fiona marina*. Therefore, none of the neurons ingressing from the cephalic plate in opisthobranchs can be homologous to pleural neurons ingressing from post-trochal ectoderm in other gastropods.

The ectodermal proliferation placode for pleural neurons must be located within the cephalopedal mass, rather than the visceropallial mass, because the pleural ganglia of gastropods do not show torsional displacement. The exact location of the paired ectodermal placodes for the pleural ganglia in a variety of prosobranchs has been described as: the sides of the head, base of the foot, opposite the statocysts or within the pleural groove. Even in *Littorina saxatilis*, a caenogastropod with an epiathroid adult nervous system (pleural ganglia close to cerebrals), the pleural ganglia arise from the base of the foot (Guyomarc'H-Cousin, 1974). In *Melibe*, the location described by these phrases corresponds to the post-trochal, laterocephalic placodes.

Jacob (1984), who used ^{3}H-thymidine autoradiography to study neurogenesis in *Aplysia californica*, suggests that pleural neurons arise from the velar lobes. However, the velar proliferation placode shown in her Figure 4a is clearly the large ciliated cells of the prototroch, with the subvelar ridge (metatroch) immediately below. What Jacobs (1984) interprets as velar cells migrating to the cerebral, and ultimately the pleural ganglia (see her Fig. 5a), may be the area where the prototroch arches over the mouth, closely approaching the cerebral ganglia (see Fig. 4 in the companion paper, Page, 1992). However, Jacob's figure 5b also shows ^{3}H-thymidine labelling in a protruding placode of ectodermal cells, lateral to the statocyst, that corresponds in appearance and location to a laterocephalic placode in *Melibe* larvae.

Appropriate location within post-trochal cephalopedal ectoderm is only one of several clues that help identify the ectodermal ingression site for pleural neurons in *Melibe* larvae. Another is the correlation between the onset of cellular ingression from this site and the appearance of the second pair of connectives extending in front of the statocysts from the cerebral ganglia. These connectives are clearly not the earlier formed cerebropedal connectives associated with the statocyst nerve. Furthermore, the two peripheral axon tracts extending to each laterocephalic placode branch from this second pair of connectives, which are presumably the cerebropleural connectives. These two axon tracts may correspond to the anterolateral and dorsal 'pedal' nerves described in adult *Melibe rosea* by De Vries (1963). Based on peripheral projection patterns, De Vries (1963) believed that the anterolateral nerve in *Melibe rosea* represents part of the ancestral anterior pleural nerve.

The size difference between left and right laterocephalic placodes correlates with a similar size difference between left and right dorsal 'pedal' nerves in adults of *Melibe* and other non-dorid nudibranchs. This difference, in turn, may relate to unilateral innervation of the penis by the right member of this nerve pair. Similarly, Régondaud (1961) noted that the right-side ectodermal placode for generating pleural neurons in *Lymnaea stagnalis* is larger than that of the left side. The pleural ganglia in *Lymnaea* are associated with the visceral loop and do not fuse with other ganglia during development.

I cannot readily explain the slight timing differences for developmental events involving the left and right laterocephalic placodes. Possibly, the temporal asymmetries are somehow related to the size difference between the two placodes and the unilateral innervation of the penis from the right side. Whatever the reason, similar developmental asynchronies involving bilaterally homologous structures are not unusual among mollusks, even for structures not affected by torsion. Examples include the cephalic tentacles of some prosobranchs, the statocysts of *Buccinum*, and the ctenidia of the bivalve, *Ostrea* (reviewed by Moor, 1983).

The slight temporal differences for developmental events involving the left and right laterocephalic placodes are dwarfed by the much greater difference between the onset of neuronal ingression from the pedal placodes and that from the laterocephalic placodes. Presumptive neu-

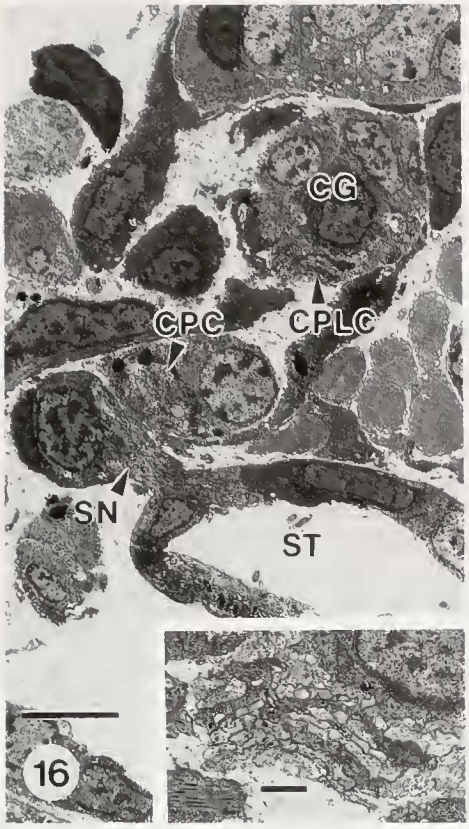
Figures 16 to 19. Series of frontal sections through left side of larva at onset of mantle retraction showing labial ganglion developing from left laterocephalic placode.

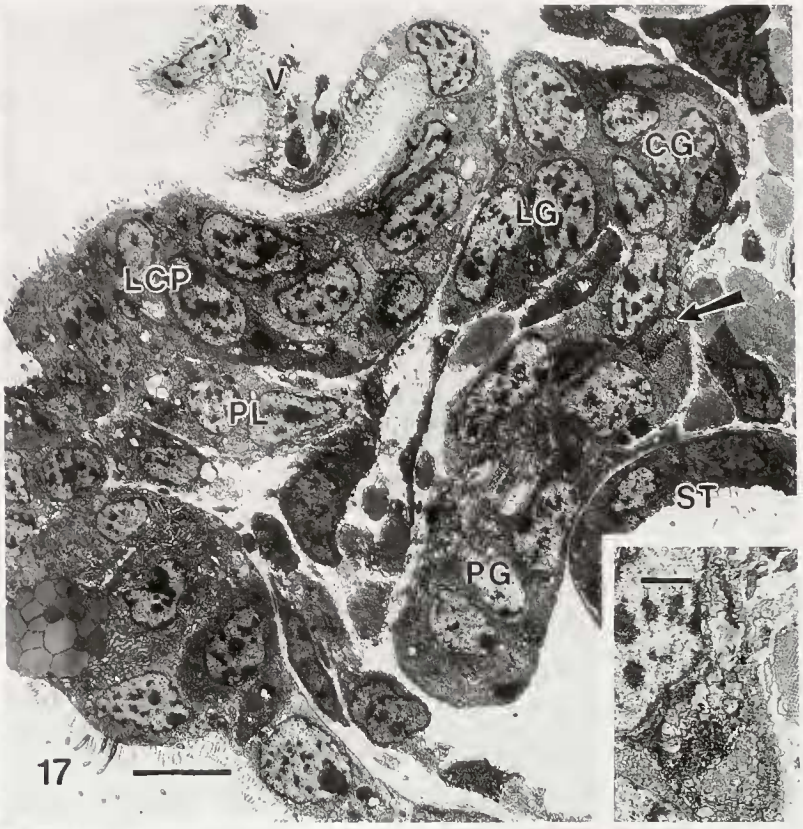
Figure 16. Cerebropedal connective (CPC) associated with statocyst nerve (SN), and cerebropleural connective (CPLC; enlarged in inset) still within anteroventral extremity of cerebral ganglion (CG). CP = cephalic plate; ST = statocyst. Scale 5 μm; inset 0.5 μm.

Figure 17. Subsequent section showing left LCP and labial ganglion (LG) projecting laterally from cerebral ganglion (CG). Arrow indicates cerebropleural connective, which is enlarged in inset. Ingressing pleural neurons (PL) are enlarged in Figure 18. PG = pedal ganglion; ST = statocyst; V = velar lobe. Scale 5 μm; inset 0.5 μm.

Figure 18. Ingressing pleural neurons (PL) associated with axons (arrowheads). Scale 5 μm.

Figure 19. Arrow indicates mitotic labial ganglion neuron ingressing from LCP. CG = cerebral ganglion; PG = pedal ganglion; ST = statocyst; V = velar lobe. Scale 5 μm.


CG
CPC
CPLC
SN
ST
16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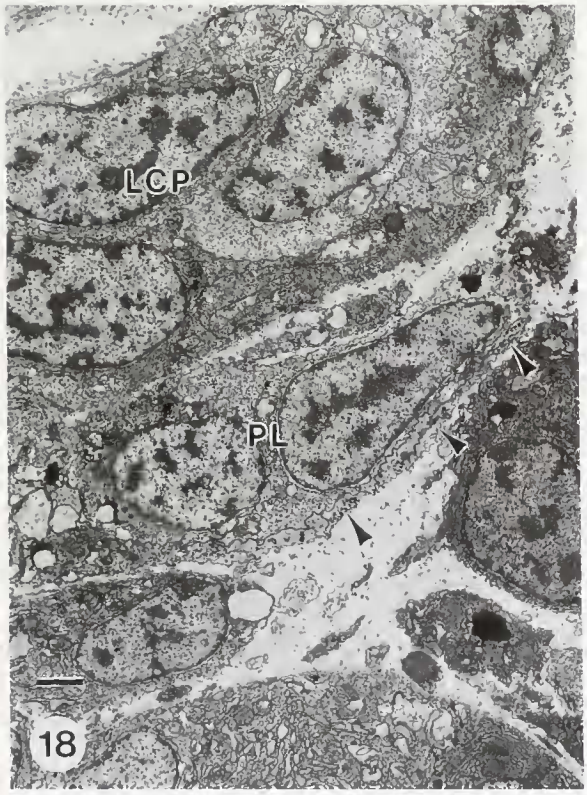
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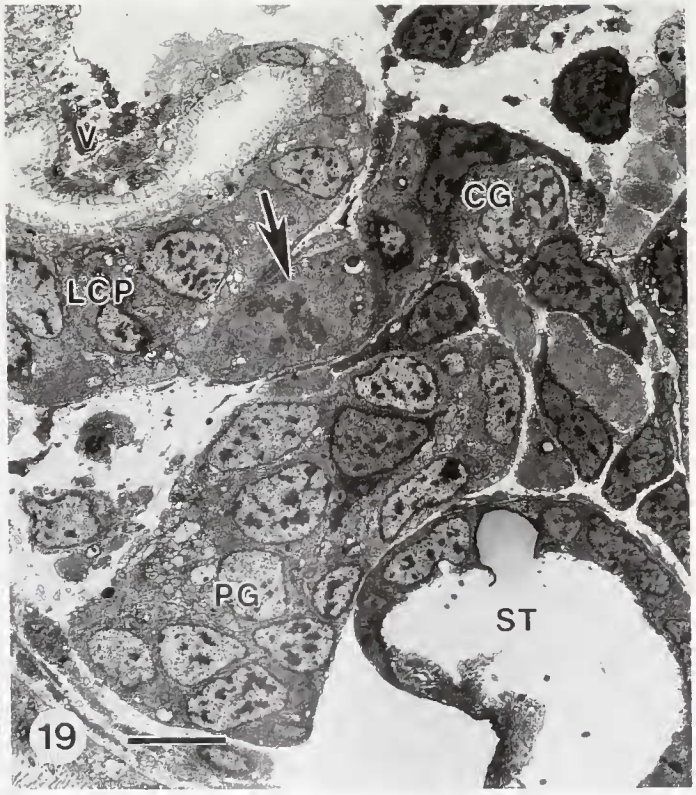
V
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19

rons begin to ingress from the pedal placodes even before hatching, whereas laterocephalic neurons do not begin to leave the ectoderm until the latter part of the shell-secreting phase. This marked difference further attests to the separate identity of neurons arising from laterocephalic and pedal placodes.

The notion that the pleural ganglia are primarily pallial in nature has become rather entrenched in the malacological literature. Although these ganglia certainly contribute to the innervation of the mantle in adult gastropods, they are not derived developmentally from visceropallial ectoderm, and Crofts (1937) observed that the pleural nerves ("external pallial nerves") extending into the mantle folds in *Haliotis* are not established until the long-lasting second phase of torsion is complete. If this were not the case, the trajectory of these nerves would be skewed by torsion.

If my arguments are accepted for the identity of the visceral loop fiber tract and associated ganglia (Page, 1992), and for the pleural ganglia in *Melibe* larvae, then one must also accept a conclusion that is unprecedented in previous developmental or neuroanatomical studies of gastropods: the cell bodies of the pleural neurons in *Melibe* have become uncoupled from the visceral loop. This conclusion is actually not so startling, because the visceral loop appears to be established by axons extending from the cerebral ganglia, with other ganglia applied later. A visceral loop (lateral cords), arising directly from the cerebral ganglia, is thought to be the ancestral condition of the gastropod nervous system and is exhibited by monoplacophorans, caudofoveates, solenogastres, and polyplacophorans (see Bullock, 1965; Salvini-Plawen, 1985; Wingstrad, 1985); none of these groups have pleural ganglia. In *Melibe*, and probably all gastropods, it is inappropriate to think of the visceral loop as an initiative of the pleural ganglia. Furthermore, uncoupling of pleural ganglia from the visceral loop is no less bizarre than the complex restructuring of the visceral loop and ganglia that occurs during development of ampullarid prosobranchs (Honegger, 1974; Demian and Yousif, 1975).

Circumstantial support for the reality of pleuropedal ganglia among other dendronotid nudibranchs comes from neuroanatomical and neurophysiological data on *Tritonia diomedea* and *T. hombergi* (Willows *et al.*, 1973; Dorsett, 1974), and on *Armina californica* (Dorsett, 1978). The dorsum and branchial tufts (cerata) of tritoniids are derived from larval mantle fold ectoderm (Thompson, 1962; Kempf and Willows, 1977). Mantle fold ectoderm also gives rise to the intestinal and visceral ganglia, which fuse to the cerebral ganglia, so it is not surprising that some neurons effecting branchial tuft withdrawal are located within the lobes projecting posteriorly from the cerebral ganglia (traditionally called the pleural lobes). However, two other effector neurons for branchial tufts are located in each 'pedal' ganglion. Extrapolating from the fact that axons of pleural neurons project into pallial tissues in shelled gastropods, it is possible that these neurons have a pleural ganglion ancestry. It is interesting to note that one of these neuronal pairs (L and RPd1), particularly that of the right side, is occasionally found within the ipsilateral cerebral ganglionic mass; that is, in the expected position if pleural ganglia were not uncoupled from the visceral loop. During the development of *Melibe*, I found that both the visceral loop and the pedal ganglia are positioned equidistant from the laterocephalic placodes in 6-day-old larvae. A relatively minor developmental miscue might easily deflect individual pleural neurons towards the visceral loop, rather than the pedal ganglion. Morphological evidence suggests that contracting muscle fibers underlying the laterocephalic placodes may pull placodal cells toward the pedal ganglion, and therefore away from the visceral loop fiber tract.

As in tritoniids, Dorsett (1978) found neurons in the pedal ganglia of *Armina californica* that control movements of the 'mantle' periphery (this species lacks cerata).

Figures 20 to 25. Series of frontal sections through left side of larva at mantle fold hypertrophy stage showing anlage of labial ganglion (LG) and trajectory of labial axons. Sections proceed distally along esophagus toward mouth.

Figure 20. Anteroventral portion of left cerebral ganglion (CG) with developing cerebrobuccal connective (large arrowheads) extending to buccal placode (BP) in ventral wall of esophagus (E). CPC = cerebropedal connective; CPLC = cerebropleural connective; PG = periphery of pedal ganglion; SN = statocyst nerve; ST = statocyst. Scale 5 μm.

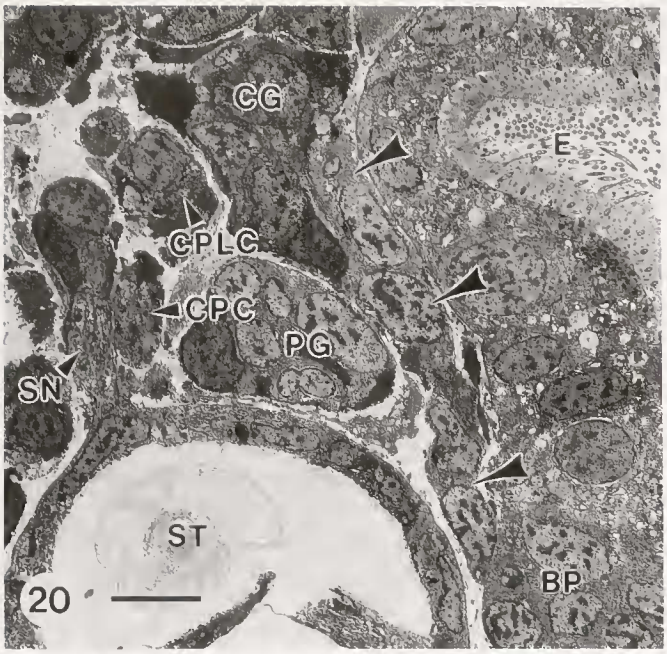
Figure 21. Cerebral ganglion end of cerebrobuccal connective showing two bundles of labial axons (arrows), one of which is extending toward axons of cerebrobuccal connective (CBC). Scale 1 μm.

Figure 22. Arrow indicates axons arising from labial ganglion neurons (LG; enlarged in Fig. 23). Note labial nerve (LN) adjacent to wall of esophagus (E). Scale 3 μm.

Figure 23. Detail from Figure 22 showing an axon (arrowheads) arising from a labial ganglion neuron (LG). Scale 0.5 μm.

Figure 24. Section passing just inside mouth showing labial ganglion (LG) continuous with laterocephalic placode (LCP). Arrowheads indicate ingressing pleural neurons. Boxed area, enlarged in Figure 25, includes labial nerve. E, esophagus; PPG = pleuropedal ganglion. Scale 5 μm.

Figure 25. Detail from Figure 24 showing labial nerve with probable synapse (arrowhead) onto esophageal cell (E). Scale 0.5 μm.


CG
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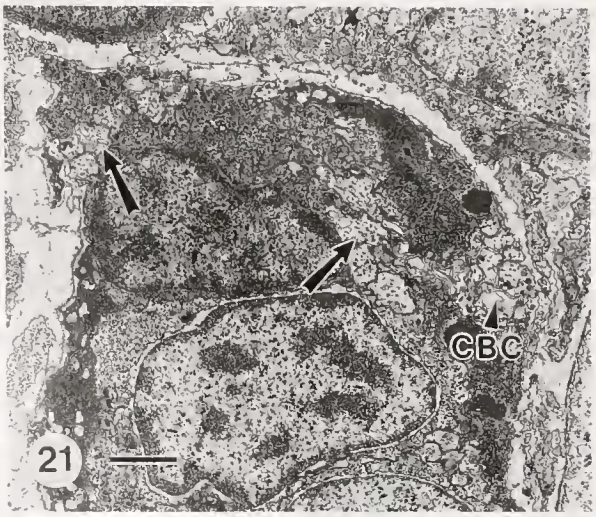
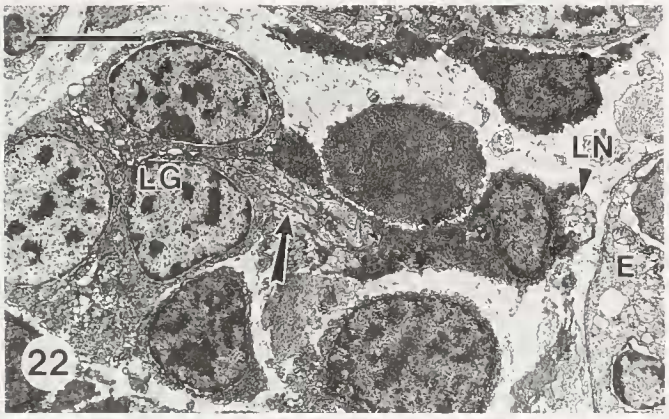
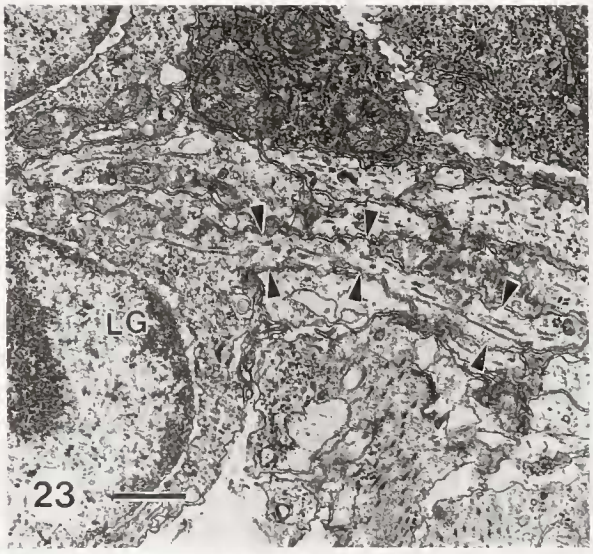

CBC
21


LG
LN
E
22


LG
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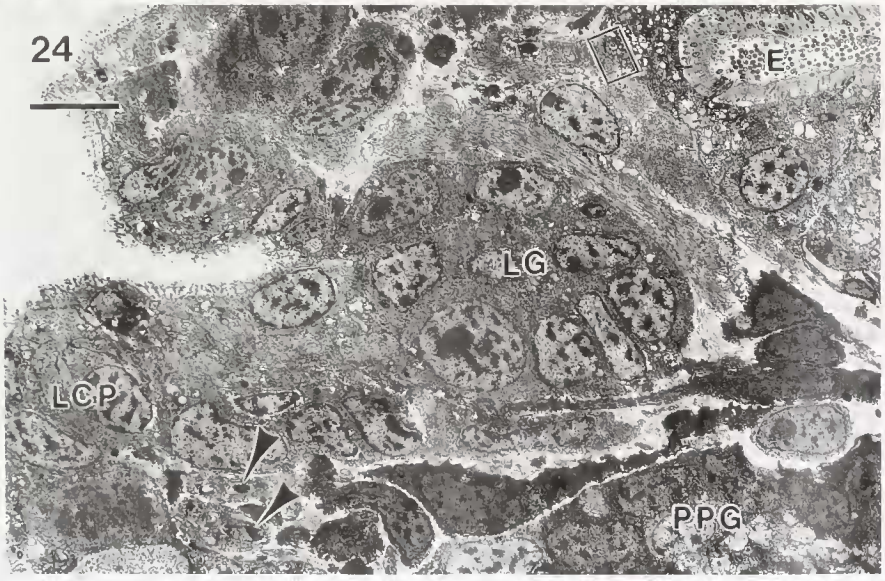
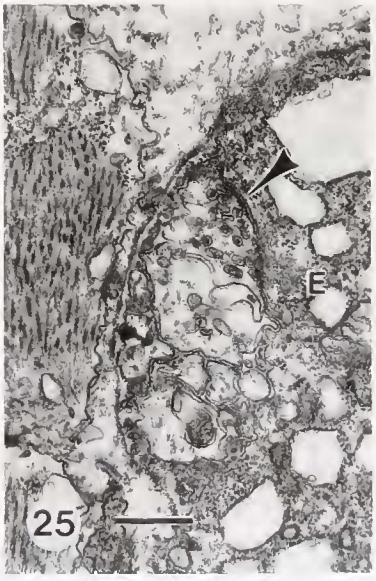

24
E
LG
LCP
PPG


E
25

Some of these, or other pedal neurons, also control movements of the foot or oral veil. These efferents can be stimulated by tactile stimuli to the foot or mantle margins.

Walters *et al.* (1983) described a conspicuous cluster of mechanosensory neurons within the undisputed pleural ganglia of *Aplysia californica*. Collectively, these primary mechanosensory neurons are sensitive to tactile stimuli applied along the entire length of the foot, including the 'parapodia' (epipodial folds). Therefore, the receptive field for pleural sensory neurons in *Aplysia* corresponds approximately to the location of larval neurogenic ectoderm for presumed pleural neurons in *Melibe*.

Labial ganglia. Unlike pleural neurons, ingressing labial neurons fuse with the ipsilateral cerebral ganglion in *Melibe*. Separate labial ganglia, which are linked beneath the buccal mass by a labial commissure and which give rise to labial nerves innervating the oral lips and buccal musculature, are found in some archaeogastropods and a pyramidellid (Fretter and Graham, 1949, 1962). They are thought to be absent or fused to the cerebral ganglia in other gastropods.

In archaeogastropods such as *Haliotis*, the cerebrobuccal connectives are routed through the labial ganglia before arriving at the buccal ganglia (see Fretter and Graham, 1962). This design can be recognized in the relationship between cerebrobuccal and labial nerve tracts in larval *Melibe*, and is the major justification for labelling these ganglia as homologues of archaeogastropod labial ganglia.

Audesirk (1979) and Audesirk and Audesirk (1980) investigated two adjacent clusters of mechanoreceptive neurons within each cerebral ganglion of *Tritonia diomedea*. The two clusters have distinct axonal projections and neurophysiological characteristics, but both are located where the labial ganglia are fused to the cerebral ganglia in *Melibe* (ventro-lateral side of each cerebral ganglion, anterior to the connectives to the pedal ganglia). One of the clusters sends axons out each ventral cerebral nerve (labelled CN4 in *Tritonia* but clearly equivalent to CN1 of *Melibe*) and also into the adjacent cerebrobuccal connective; a trajectory that is consistent with my observations on initial labial axons in *Melibe* larvae. These mechanoreceptors respond to pressure or stretch of the oral tube or jaw closer muscles (Audesirk, 1979). The second category of mechanoreceptive neurons sends axons out cerebral nerves extending to the oral veil and a pedal nerve extending to the anterior foot (Audesirk and Audesirk, 1980). Although further data are needed, I consider it very possible that cerebral nerves innervating the oral veil in *Tritonia* and the oral hood in *Melibe* are also established by labial neurons.

It might be argued that the labial ganglia are better identified as the pleural ganglia in *Melibe*. They arise from a pair of post-trochal ectodermal sites of appropriate location for pleural neurons and they fuse with the cerebral ganglia, which the traditional interpretation dictates for the pleural ganglia of opisthobranchs. However, the trajectory of axons arising from labial neurons is inappropriate for pleural neurons.

Critique of previous neurodevelopmental models

All previous accounts of sequential neurodevelopment in nudibranchs have been histological, yet this method cannot reveal with certainty all neuronal ingression sites from ectodermal proliferation placodes or trajectories of early connectives and axon tracts. Uncertainty resulting from limited resolution has resulted in three different models (more including variations) for the pattern of neurogenesis in nudibranchs. These earlier models, plus the current model generated from ultrastructural study of *Melibe leonina* larvae, are illustrated schematically in Figure 26 and are discussed below.

The groundbreaking study of nudibranch organogenesis was done by Thompson (1958) on the lecithotrophic veliger of the dorid nudibranch, *Adalaria proxima*. Thompson identified cerebral, optic, pedal, pleural, and buccal ganglia in *Adalaria* (Fig. 26A). The intestinal and visceral ganglia escaped notice, possibly because they may fuse precociously to the cerebrals in this lecithotrophic veliger. Alternatively, Tardy (1970) has suggested that Thompson misidentified visceral loop ganglia as the buccal ganglia.

According to Thompson (1958), the pleural ganglia of *Adalaria* are large neuronal masses located within the base of the foot, and they fuse with the cerebrals at metamorphosis. The 'anterolateral propodial ganglia' described by Chia and Koss (1989) and Arkett *et al.* (1989) for the dorid nudibranch *Onchidoris bilamellata* have a similar size and location to the ganglia identified as the pleurals in *Adalaria*. Further study is needed to determine if these are indeed pleural ganglia, or alternatively, the labial ganglia.

Tardy (1970, 1974) proposed that both cerebral and pleural neurons arise from the cephalic plate in *Aeolidiella alderi*, thereby producing cerebropleural ganglia that are fused from the beginning (Fig. 26B). As discussed earlier, this contradicts observations made on many other species. Tardy also recognized buccal ganglia in *Aeolidiella alderi*, but his description of their early development is highly anomolous. Many studies, including this one on *Melibe*, have found that neurogenic ectoderm for buccal ganglia is located within the ventral wall of the distal esophagus (Smith, 1935; Creek, 1951; D'Asaro, 1969; Guyomarc'H-Cousin, 1974; Honegger, 1974; Demian and Yousif, 1975), but Tardy (1974; p. 315 and Fig. 2E) shows the location of buccal neurogenic ectoderm as lateral to the mouth, proximal to the pedal placodes, and adjacent to the statocysts. I suggest that these are the laterocephalic placodes. Tardy also described a pair of ganglia that arise from the ectoderm of the oral tentacles, but only after metamorphosis.

Having rejected Tardy's (1970, 1974) interpretation for the origin of pleural ganglia in nudibranchs, my colleagues and I identified the ganglia directly behind the cerebrals as the pleural ganglia in previous histological studies on the dorid *Doridella steinbergae* (Bickell and Chia, 1979) and on *Melibe leonina* (Bickell and Kempf, 1983). Kempf *et al.* (1987) gave the same interpretation for the larval CNS of the dendronotid *Tritonia diomedea*, except they also resolved a visceral ganglion (Fig. 26C). This model is superficially attractive because it produces an adult CNS with ganglionic regions that conform to the traditional interpretation based on comparative neuroanatomical studies of adult opisthobranchs (Fig. 27A). However, this model fails to explain why the left 'pleural ganglion' in the larva projects ventrally relative to the right, when pleural ganglia should not be involved in torsional displacements. I now believe that these ganglia are the intestinals.

Figure 26D shows my current interpretation of ganglionic regions within the late larval nervous system of *Melibe leonina*. The subsequent pattern of ganglionic fusions produces a CNS with ganglionic regions that differ from the traditional interpretation. The differences are illustrated in Figure 27.

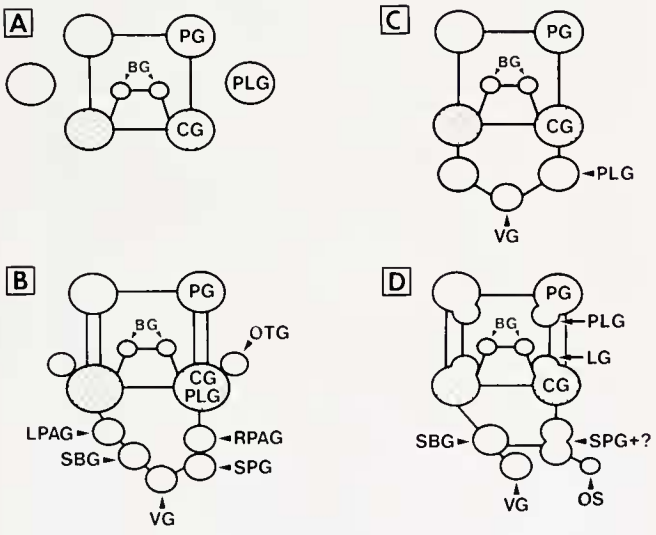


Figure 26. Four interpretations of the developing nudibranch CNS. Relative lengths of connectives and sizes of ganglia are not accurate; optic and rhinophoral ganglia not shown. (A) Thompson (1958) for *Adalaria proxima*. (B) Tardy (1970, 1974) for *Acolidiella alderi*. (C) Bickell and Chia (1979) for *Doridella steinbergae* and Bickell and Kempf (1983) for *Melibe leonina*; visceral ganglion added by Kempf *et al.* (1987) for *Tritonia diomedea*. (D) present study on *Melibe leonina*. Abbreviations: BG = buccal ganglia; CG = cerebral ganglion; LG = labial ganglion; LPAG = left parietal ganglion; OS = osphradial neurons; OTG = oral tentacle ganglion; PG = pedal ganglion; PLG = pleural ganglion; RPAG = right parietal ganglion; SBG = subintestinal ganglion; SPG = supraintestinal ganglion; VG = visceral ganglion.

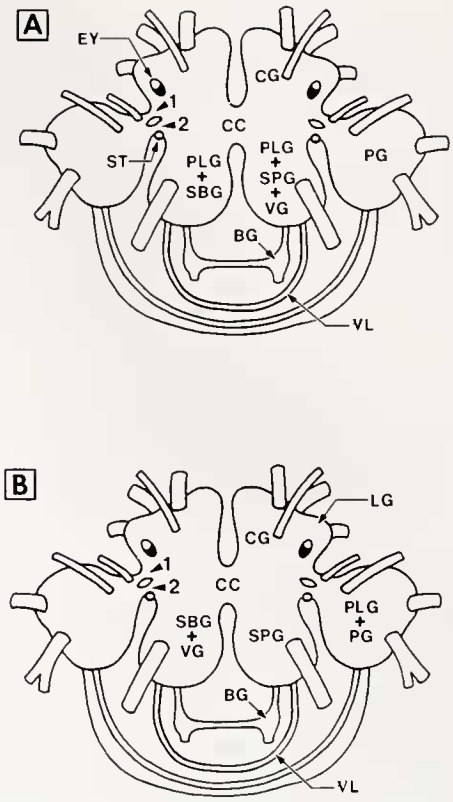


Figure 27. Interpretations of ganglionic regions in adult CNS of *Melibe leonina* (optic and rhinophoral ganglia not shown). (A) Traditional Model. Arrowhead no. 1 indicates cerebropedal connective; arrowhead no. 2 indicates pleuropedal connective. (B) Revised Model. Arrowhead no. 1 indicates cerebropleural connective; arrowhead no. 2 indicates cerebropedal connective. BG = buccal ganglion; CC = cerebral commissure; CG = cerebral ganglion; EY = eye; LG = labial ganglion (ventrally located); PG = pedal ganglion; PLG = pleural ganglion; SBG = subintestinal ganglion; SPG = supraintestinal ganglion; VG = visceral ganglion; VL = visceral loop.

Thoughts on phylogeny

Questions about phyletic relationships among opisthobranchs and about opisthobranch origins continue to be debated. Uncertainties stem from the large degree of morphological variation within the group and many instances of apparent parallelism (Ghiselin, 1965; Gosliner, 1981, 1991; Gosliner and Ghiselin, 1984). Nervous systems have a reputation for conservatism and are therefore valued as phyletic characters. It might be hoped that opisthobranch nervous systems could help unmask relationships otherwise hidden by superficial morphological differences or convergences. To date, proposed phylogenies have considered only adult neuroanatomical characters, with emphasis given to the length and detorsion of the visceral loop, and extent of ganglionic fusions. Using the latter criterion, Russell (1929) argued convincingly that nudibranchs are unlikely ancestors for sacoglossans. Nevertheless, Ghiselin (1965), among others, believes that

most of these characters show polyphyletic trends, and the extensively fused nervous systems of many adult opisthobranchs offer few other unambiguous clues to assist in phylogenetic reconstructions. However, ultrastructural study of neurodevelopment has revealed much more about an opisthobranch nervous system than is apparent from adult CNS structure. Indeed, the new developmental data about homologous ganglionic regions in *Melibe* raise serious questions about the validity of adult-based neuroanatomical interpretations given for other opisthobranchs.

In a revision of gastropod systematics, Salvini-Plawen and Haszprunar (1987) and Haszprunar (1988) argued that a hypoathroid or dystenoid nervous system (fused or semi-fused pleural and pedal ganglia) is a diagnostic character for the archaeogastropod grade. Based partly on the assumption that opisthobranch pleural ganglia are fused to the cerebrals (epiathroid nervous system; typical of caenogastropods), when not distinct in adults, Haszprunar (1985, 1988) proposed a primitive caenogastropod ancestor for the Opisthobranchia. Gosliner (1981) came to the same conclusion based on a suite of many criteria. It is therefore surprising to find a hypoathroid nervous system in a nudibranch, although the pleural ganglia of *Aplysia californica* are also very close to the pedal ganglia (well illustrated by fig. 4 of Cash and Carew, 1989). These observations contradict Haszprunar's proposed criterion for the archaeogastropod grade and undermine the notion of an epiathroid nervous system for all opisthobranchs. The possibility that the condition in *Melibe* is merely an exceptional, secondarily derived state, possibly related to the unusual oral hood of this genus, must be determined.

I have identified pleural neurons in *Melibe*, partly on the assumption that the two connectives between 'cerebral' and 'pedal' ganglia are cerebropedal and cerebro-pleural-pedal connectives. This dictum for gastropod CNS organization is supported by a large body of comparative anatomical data, but its universality is certainly not proven. Again, ultrastructural studies of development for a variety of gastropod groups are needed.

To date, labial ganglia have been identified in adults of some archaeogastropods (reviewed by Fretter and Graham, 1962), a pyramidellid (Fretter and Graham, 1949; pyramidellids have strong opisthobranch affinities), and in larvae of the nudibranch *Melibe leonina*. However, if the subcerebral commissure is evidence of labial ganglia fused to cerebral ganglia, as suggested by Bullock (1965), then labial ganglia are present also in other opisthobranchs and in pulmonates. Neither labial ganglia nor a subcerebral commissure have been found among caenogastropods, nor have they been reported in developmental stages of this group.

In conclusion, the nervous system of *Melibe* shares major plesiomorphous characters (pleuropedal ganglia, labial ganglia) with that of archaeogastropods. Although the uncoupling of the pleural ganglia from the visceral loop is an apomorphy not suspected previously for any other gastropod, the *Melibe* nervous system is not readily derived from the epiathroid nervous system typical of extant caenogastropods. The validity of these speculations must await further comparative studies.

Acknowledgments

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Literature Cited

Arkett, S. A., F. S. Chia, J. I. Goldberg, and R. Koss. 1989. Identified settlement receptor cells in a nudibranch veliger respond to specific cue. *Biol Bull* **176:** 155–160.

Audesirk, T. E. 1979. Oral mechanoreceptors in *Tritonia diomedea* 1. Electrophysiological properties and location of receptive fields. *J. Comp. Physiol.* **A130:** 71–78.

Audesirk, G., and T. E. Audesirk. 1980. Complex mechanoreceptors in *Tritonia diomedea* 1. Responses to mechanical and chemical stimuli. *J. Comp. Physiol.* **A141:** 101–109.

Bickell, L. R., and F. S. Chia. 1979. Organogenesis and histogenesis in the planktotrophic veliger of *Doridella steinbergae* (Opisthobranchia: Nudibranchia). *Mar. Biol.* **52:** 291–313.

Bickell, L. R., and S. C. Kempf. 1983. Larval and metamorphic morphogenesis in the nudibranch *Melibe leonina* (Mollusca: Opisthobranchia). *Biol. Bull.* **165:** 119–138.

Boettger, C. 1954. Die Systematik der euthyneuren Schnecken. *Zool. Anz. Leipzig* (suppl.) **17:** 253–279.

Bullock, T. H. 1965. The Mollusca. Pp. 1273–1515 in *Structure and Function in the Nervous Systems of Invertebrates*, V. 2, T. H. Bullock and G. A. Horridge, eds. Freeman Press, San Francisco.

Cash, D., and T. J. Carew. 1989. A quantitative analysis of the development of the central nervous system in juvenile *Aplysia californica*. *J. Neurobiol.* **20:** 25–47.

Casteel, D. B. 1904. The cell-lineage and early larval development of *Fiona marina*, a nudibranch mollusc. *Proc. Acad. Nat. Sci. Philadelphia* **56:** 325–405.

Chia, F. S., and R. Koss. 1989. The fine structure of a newly discovered propodial ganglia of the veliger larva of the nudibranch *Onchidoris bilamellata*. *Cell Tissue Res.* **256:** 17–26.

Conklin, E. G. 1897. The embryology of *Crepidula*. *J. Morphol.* **13:** 1–226.

Creek, G. A. 1951. The reproductive system and embryology of the snail *Pomatias elegans* (Muller). *Proc. Zool. Soc. Lond.* **121:** 599–640.

Crofts, D. R. 1937. The development of *Haliotis tuberculata*, with special reference to the organogenesis during torsion. *Phil. Trans. R. Soc. Lond.* **B228:** 219–268.

Cumin, R. 1972. Normentafel zur Organogenese von *Limnaea stagnalis* (Gastropoda, Pulmonata) mit besonderer Berücksichtigung der Mitteldarmdrüse. *Rev. Suisse Zool.* **79:** 709–774.

D'Asaro, C. N. 1969. The comparative embryogenesis and early organogenesis of *Bursa corrugata* and *Distorsia clarthrata*. *Malacologia* **9:** 349–389.

Demian, E. S., and F. Yousif. 1975. Embryonic development and organogenesis in the snail *Marisa cornuarietis* (Mesogastropoda: Am-

pullariidae). V. Development of the nervous system. *Malacologia* **15:** 29–42.

De Vries, J. B. 1963. Contribution to the morphology and histology of the nudibranch *Melibe rosea* Rang. *Ann. Univ. Stellenbosch* **38** (ser. A): 105–153.

Dorsett, D. A. 1974. Neuronal homologies and the control of branchial tuft movements in two species of *Tritonia*. *J. Exp. Biol.* **61:** 639–654.

Dorsett, D. A. 1978. Neurons controlling foot and mantle movements in *Armina californica*. *Mar. Behav. Physiol.* **5:** 307–324.

Dorsett, D. A. 1986. Brains to cells: the neuroanatomy of selected gastropod species. Pp. 101–187 in *The Mollusca*, K. M. Wilbur, ed. V.9, *Neurobiology and Behavior*, part 2, A. O. D. Willows, ed. Academic Press, New York.

Fretter, V., and A. Graham. 1949. The structure and mode of life of the Pyramidellidae, parasitic opisthobranchs. *J. Mar. Biol. Assoc. U.K.* **28:** 493–532.

Fretter, V., and A. Graham. 1962. *British Prosobranch Molluscs: Their Functional Anatomy and Ecology*. Ray Society, London.

Ghiselin, M. 1965. Reproductive function and the phylogeny of opisthobranch gastropods. *Malacologia* **3:** 327–378.

Gosliner, T. M. 1981. Origins and relationships of primitive members of the Opisthobranchia (Mollusca: Gastropoda). *Biol. J. Linn. Soc.* **16:** 197–225.

Gosliner, T. M. 1987. A review of the genus *Melibe* (Opisthobranchia; Dendronotacea) with descriptions of two new species. *Veliger* **29:** 400–414.

Gosliner, T. M. 1991. Morphological parallelism in opisthobranch gastropods. *Malacologia* **32:** 313–327.

Gosliner, T., and M. Ghiselin. 1984. Parallel evolution in opisthobranch gastropods and its implications for phylogenetic methodology. *Syst. Zool.* **33:** 255–274.

Guiart, J. 1901. Contribution à l'étude des gastéropodes opisthobranchs et en particulier de céphalaspides. *Mem. Soc. Zool. Fr.* **14:** 5–219.

Guyomarc'H-Cousin, C. 1974. Étude descriptive de l'organogénèse du système nerveux chez *Littorina saxatilis* (Olivi) Gastéropode Prosobranche. *Ann. Embryol. Morphog.* **4:** 349–364.

Haszprunar, G. 1985. The Heterobranchia—a new concept of the phylogeny of the higher Gastropoda. *Z. Zool. Syst. Evol.* **23:** 15–37.

Haszprunar, G. 1988. On the origin and evolution of major gastropod groups with special reference to the Streptoneura. *J. Moll. Stud.* **54:** 367–441.

Hickmott, P. W., and T. J. Carew. 1991. An autoradiographic analysis of neurogenesis in juvenile *Aplysia californica*. *J. Neurobiol.* **22:** 313–326.

Hoffmann, H. 1936. Opisthobranchia. Pp. 641–864 in *Klassen und Ordungen des Tierreichs*, v.3, H. G. Bronns, ed. Acad. Verlagsges., Leipzig.

Honegger, T. 1974. Die Embryogenese von *Ampullarius* (Gastropoda, Prosobranchia). *Zool. Jahrb. Abt. Anat. Ontogenie Tiere* **93:** 1–76.

Hurst, A. 1968. The feeding mechanism and behaviour of the opisthobranch *Melibe leonina*. *Symp. Zool. Soc. Lond.* **22:** 151–166.

Jacob, M. H. 1984. Neurogenesis in *Aplysia californica* resembles nervous system formation in vertebrates. *J. Neurosci.* **4:** 1225–1239.

Kandel, E. R. 1979. *Behavioral Biology of* Aplysia. Freeman, San Francisco.

Kempf, S. C., B. Masinovsky, and A. O. D. Willows. 1987. A simple neuronal system characterized by a monoclonal antibody to SCP neuropeptides in embryos and larvae of *Tritonia diomedea* (Gastropoda, Nudibranchia). *J. Neurobiol.* **18:** 217–236.

Kempf, S. C., and A. O. D. Willows. 1977. Laboratory culture of the nudibranch *Tritonia diomedea* Bergh (Tritoniidae: Opisthobranchia) and some aspects of its behavioral development. *J. Exp. Mar. Biol. Ecol.* **30:** 261–276.

McAllister, L. B., R. H. Scheller, E. R. Kandel, and R. Axel. 1983. *In situ* hybridization to study the origin and fate of identified neurons. *Science* **222:** 800–808.

Moor, B. 1983. Organogenesis. Pp. 123–177 in *The Mollusca*, K. M. Wilbur, ed., v.3, *Development*, N. H. Verdonk, J. A. M. van den Biggelaar, and A. S. Tompa, eds. Academic Press, New York.

Page, L. R. 1992. New interpretation of a nudibranch central nervous system based on ultrastructural analysis of neurodevelopment in *Melibe leonina* I. Cerebral and visceral loop ganglia. *Biol. Bull.* **182:** 348–365.

Raven, C. P. 1958. *Morphogenesis: The Analysis of Molluscan Development*. Pergamon Press, Oxford.

Raven, C. P. 1975. Development. Pp. 367–400 in *Pulmonates*, Vol. 1, V. Fretter and J. Peake, eds. Academic Press, New York.

Régondaud, J. 1961. Formation due système nerveux et torsion chez *Lymnaea stagnalis* L. (Mollusque Gastéropode). *C.R. Hebd. Seances L'Acad. Sci.* **252:** 1203–1205.

Régondaud, J. 1964. Origine embryonnaire de la cavité pulmonaire de *Lymnaea stagnalis* L. Considérations particulières sur la morphogénèse de la commissure viscérale. *Bull. Biol. Fr. Belg.* **98:** 433–471.

Russell, L. 1929. The comparative morphology of the elysioid and aeolidioid types of the molluscan nervous system, and its bearing on the relationships of the ascoglossan nudibranchs. *Proc. Zool. Soc. Lond.* **14:** 197–233.

Salvini-Plawen, L. v. 1985. Early evolution and the primitive groups. Pp. 59–150 in *The Mollusca*, K. M. Wilbur, ed., V.10, *Evolution*, E. R. Trueman and M. R. Clarke, eds. Academic Press, New York.

Salvini-Plawen, L. v., and G. Haszprunar. 1987. The Vetigastropoda and the systematics of the streptoneurous gastropods (Mollusca). *J. Zool. Lond.* **A211:** 747–770.

Schmekel, L. 1985. Aspects of evolution within the Mollusca. Pp. 221–267 in *The Mollusca*, K. M. Wilbur, ed., v.10, *Evolution*, E. R. Trueman and M. R. Clarke, eds. Academic Press, New York.

Smith, F. G. W. 1935. The development of *Patella vulgata*. *Phil. Trans. R. Soc. Lond.* **B225:** 95–125.

Smith, S. T. 1967. The development of *Retusa obtusa* (Montagua) (Gastropoda, Opisthobranchia). *Can. J. Zool.* **45:** 737–764.

Tardy, J. 1970. Contribution à l'étude des métamorphoses chez les nudibranches. *Ann. Sci. Nat., Zool., Paris* **12:** 299–370.

Tardy, J. 1974. Morphogenèse du système nerveux chez les mollusques nudibranches. *Haliotis* **4:** 61–75.

Thompson, T. E. 1958. The natural history, embryology, larval biology, and post-larval development of *Adalaria proxima* (Alder and Hancock) (Gastropoda, Opisthobranchia). *Phil. Trans. R. Soc. Lond.* **B242:** 1–58.

Thompson, T. E. 1962. Studies on the ontogeny of *Tritonia hombergi* Cuvier (Gastropoda Opisthobranchia). *Phil. Trans. R. Soc. Lond.* **B245:** 172–218.

Verdonk, N. H., and J. A. M. van den Biggelaar. 1983. Early development and the formation of the germ layers. Pp. 91–122 in *The Mollusca*, K. M. Wilbur, ed., v.3, *Development*, N. H. Verdonk, J. A. M. van den Biggelaar, and A. S. Tompa, eds. Academic Press, New York.

Walters, E. T., J. H. Byrne, T. J. Carew, and E. R. Kandel. 1983. Mechanoafferent neurons innervating tail of *Aplysia*. I. Response properties and synaptic connections. *J. Neurophysiol.* **50:** 1522–1542.

Willows, A. O. D., ed. 1985–1986. *The Mollusca*, K. M. Wilbur, ed. v.8 and 9, *Neurobiology and Behavior*, parts 1 and 2. Academic Press, New York.

Willows, A. O. D., D. A. Dorsett, and G. Hoyle. 1973. The neuronal basis of behavior in *Tritonia*. I. Functional organization of the nervous system. *J. Neurobiol.* **4:** 207–237.

Wingstrad, K. G. 1985. On the anatomy and relationships of recent Monoplacophora. *Galathea Rep.* **16:** 7–94.